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Assessment of antiviral therapeutics in animal models of Lassa fever

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Lassa virus (LASV) is an emerging zoonotic virus endemic in West Africa that can cause severe haemorrhagic Lassa fever (LF) in humans. LF recently gained international attention as a prominent infectious disease, leading to increasingly severe outbreaks in Nigeria over the past three years. Morbidity and mortality associated with LF disease in Nigeria continue to rise with 106 deaths reported in 2016, 143 in 2017 and 562 in 2018. Despite the significant health impact LF imposes on West Africa there are currently no FDA-approved therapeutics or vaccines available for treatment and prevention. This review focuses on the assessment and current state of LF antiviral therapeutics in animal models and their potential role in reducing disease burden throughout West Africa.

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Introduction

LASV, a zoonotic single-stranded, bi-segmented negative sense RNA virus belonging to the Arenavirus family causes severe haemorrhagic LF disease in humans with a fatality rate ranging from 1% to as high as 30% during outbreak situations [1]. LF is endemic to West Africa, where seasonal outbreaks in Nigeria have been growing in intensity over the past three years [1]. It is estimated that 300 000–500 000 cases occur on an annual basis making LF the most prominent haemorrhagic disease in Africa [2•]. Currently there are no FDA-approved LASV-specific antiviral therapeutics or drugs available for the treatment of LF. LASV infections occur when humans come into contact with contaminated rodent urine or feces. Human–human transmission and nosocomial infections have also been reported via direct contact with contaminated body fluids or tissues [3]. The Nigerian Centre for Disease Control (NCDC), has reported 633 confirmed cases in 2018 and 526 confirmed cases in 2019 as of March 31st [4,5]. Genomic analysis of 2018 LASV isolates has suggested a wide range of viral diversity according to geographic location [2•]. However, it is unknown if the severity and scale of recent outbreaks are influenced by a combination of seasonal climatic changes affecting the rodent reservoir (*Mastomys natalensis*) or improved Nigerian surveillance and diagnostics. The significant genomic heterogeneity and geographical range of LASV highlight the importance of improved animal models of LASV disease for the testing of medical countermeasures. The development of therapeutics and antivirals with sound pre-clinical data justifying further clinical advancement will be critically important for the future treatment of LF. This review focuses on the therapeutic efficacy of antiviral compounds using animal models and their potential for further clinical development.

Current animal models for the development of therapeutics against LASV infection

Animal models of infection have become important tools for evaluating potential antiviral compounds and therapeutics. Animal models such as mice, guinea pigs, ferrets, and non-human primates (NHPs) have typically been utilized; however, some of these models often fail to recapitulate human disease (Table 1). The majority of animal models developed have used the prototypic LASV strain, Josiah (clade IV), originating from Sierra Leone [10]. However, the applicability of using Josiah as a lethal model may not be the most ideal given that it does not represent the overall disease burden in West Africa and Nigeria. Animal models that represent circulating clade II and III viruses are urgently needed for LASV research and therapeutic development. Here we outline the animal models that have been used to identify countermeasures against LASV.

Immuno-deficient mice

Mice lacking either the transcription factor STAT-1 (STAT-1$^{-/-}$ mice) or type I interferon receptor (IFNAR$^{-/-}$) are rendered immunocompromised due the inability to respond accordingly to certain immune signals and innate cytokines [6,7]. These mice are more vulnerable to viral infection and have been used as an animal model for several viruses including LASV. Most strains of wild type mice have been shown to be resistant to LASV infection; however, Yun *et al.* were able to show that IFNAR$^{-/-}$ mice as well as IFN-a/bgR$^{-/-}$ are susceptible to LASV infection resulting in high viral

Table 1

Animal models of LASV infection

Animal	Strain/species	Challenge strain	Dose and route	Survival	Ref.
Mouse	IFNAR$^{-/-}$ (129 Bkgrd.)	Josiah	10^4 PFU i.p.	100%	[8]
	IFN-α/βgR$^{-/-}$ (129 Bkgrd.)	Josiah	10^4 PFU i.p.	100%	[8]
	Chimeric IFNAR$^{-/-}$ (C57bl/6 Bkgrd.)	Ba366	10^3 FFU i.p.	0%	[9]
	IFNAR$^{-/-}$ (129 Bkgrd.)	AV	10^3, 10^5 FFU i.v.	100%	[10]
		Ba366	10^3 FFU i.v.	100%	[10]
		Nig04-10	10^3 FFU i.v.	100%	[10]
		Josiah	10^3 FFU i.v.	100%	[10]
	STAT1$^{-/-}$	rJosiah	10^4 PFU i.p.	0%	[11]
Guinea pig		Josiah	2, 24, 2400, 2.4×10^5 PFU s.c.	0%	[12]
	Strain 13	Josiah	10^4 $TCID_{50}$ i.p.	0%	[16]
		Z-132	10^4 $TCID_{50}$ i.p.	0%	[16]
		R-Soromba	10^4 $TCID_{50}$ i.p.	43%	[16]
	Hartley	Josiah	2, 24, 2400, 2.4×10^5 PFU s.c.	70%	[12]
		GPA-Josiah	10^5 $TCID_{50}$ i.p.	0%	[14••]
Ferret	*Mustela putorius*	Josiah	1×10^4 $TCID_{50}$ i.m. or i.n.	100%	[a]
NHP (Macaque)	Rhesus	Josiah	$10^{5.1}$ PFU s.c.	47%	[18]
		Josiah	$10^{6.1}$ PFU s.c.	40%	[20••]
	Cynomolgus	Josiah	10^4 $TCID_{50}$ i.p.	0%	[16]
		Z-132	10^4 $TCID_{50}$ i.p.	0%	[16]
		R-Soromba	10^4 $TCID_{50}$ i.p.	33%	[16]
		Josiah	3000 PFU i.m.	0%	[17]
		Josiah	10^4 $TCID_{50}$ i.m.	0%	[27•]
		Josiah	$10^{6.1}$ PFU s.c.	7%	[22]

i.p = Intraperitoneal; s.c = Subcutaneous; i.m = Intramuscular; i.n = Intranasal; FFU = Focus forming units; PFU = plaque-forming units; GPA-LASV = Guinea pig-adapted; $TCID_{50}$ = 50% tissue culture infectious dose.

titers, although this did not result in a lethal phenotype [8]. Ostereich *et al.* expanded upon this model by developing IFNAR$^{-/-}$ mice with chimeric wild type immune systems. Subsequent LASV infection is 100% lethal in these animals, and this model has been used to assess the efficacy of favipiravir and ribavirin against lethal LASV infection [9]. Additionally, it has been shown that IFNAR$^{-/-}$ mice infected with several strains of LASV reproduce similar signs of disease, reflecting the utility of this model for testing therapeutics against a wide array of viral strains [10]. The limitations of these mouse models include the incomplete lethality in IFNAR mice and the need to generate bone marrow chimeric animals to achieve lethality. Yun *et al.* showed that mice deficient in STAT-1 were highly susceptible to LASV infection, and this model may provide an alternate means for testing anti-viral compounds in a mouse model of LASV infection [11]. Despite certain limitations these mice are able to provide a useful small animal model for the screening of potential therapeutics and anti-viral compounds.

Guinea pigs

Currently, guinea pigs are the most practical small animal model to study the pathogenesis of LF. While NHPs remain the gold standard for pre-clinical efficacy studies, guinea pigs have been a useful model for understanding pathogenesis of LASV infection and for testing different clinical countermeasures. For the classical strain Josiah, uniformly lethal infection occurs in strain 13 guinea pigs, whereas infection of outbred Hartley guinea pigs produces a sublethal infection [12]. Infected guinea pigs have high viral titers in all extraneural tissues with the highest levels of virus found in the lungs, spleen, pancreas, lymph nodes, renal gland, kidneys, salivary gland, liver, and heart [12]. The main difference in the pathophysiology of LASV in guinea pigs compared to humans is the manifestation of respiratory and cardiac signs of disease. Guinea pigs develop respiratory insufficiency including pulmonary edema and myocarditis leading to death, while human disease mainly targets the liver resulting in degeneration of hepatocytes and hepatic necrosis [13]. Despite this difference, the guinea pig model has been used extensively to test various therapeutics and vaccines for LASV. Given that the acquisition of inbred strain 13 guinea pigs can be a logistical challenge and that outbred Hartley guinea pigs are resistant to infection, Safronetz *et al.* developed a guinea pig-adapted LASV (GPA-LASV) that causes uniform lethality in Hartley guinea pigs [14••]. This newly adapted virus allows for testing of anti-viral compounds and therapeutics in a more easily accessible and common small laboratory animal and one that is also more immunologically robust due to its outbred nature [14••,15]. While this model is valuable for testing against the classic Josiah strain, assessing the efficacy of compounds against other LASV strains in Hartley guinea pigs also requires adaptation of those viruses. In the case of newly discovered or isolated LASV strains, the use of strain 13 guinea pigs is still preferable for initial characterization [16]. As a result, strain 13 and Hartley guinea

pigs have been used to initially test the efficacy of several therapeutics against LASV.

Non-human primates

Multiple species of NHPs have been used for experimental infection with LASV, including rhesus and cynomolgus macaques, marmosets, capuchin monkeys, among others [13]. LASV inoculation of NHPs has been found to reproduce several hallmarks of LF infection in humans. For rhesus and cynomolgus macaques, this includes the tissue tropism of the virus and hepatic, adrenal, and splenic necrosis [17,18]. An additional observation seen in infection of cynomolgus macaques is central nervous system involvement that mirrors what has been seen in human cases of LF [17]. Interestingly, rhesus macaques seem to be more susceptible to lower doses of challenge virus, which may be something to keep in mind when evaluating new or different strains using this model. Another feature of NHP models of disease is the ability to examine the role of the immune response during LF as the development, or lack of an appropriate cell-mediated immune response corresponds to lethal LF disease [19]. The cost and need for appropriate housing within high containment facilities make the testing of various therapeutics in NHPs difficult. However, NHPs are currently the gold standard animal model for the study of LF pathogenesis.

Antivirals for LASV treatment

Small-molecule antivirals are low molecular weight organic compounds that are biologically active. Currently a limited number of small molecules have been identified and investigated for antiviral efficacy against LASV *in vitro* and *in vivo*. These potential drugs include Ribavirin, Favipiravir, and other small molecule inhibitors, which target various stages of the virus life cycle (Table 2).

Ribavirin

Ribavirin is a guanosine analog that has shown antiviral activity against RNA and DNA viruses, and it has been suggested to be effective in preventing LF. Historical data from the 1980's, seemed to indicate that ribavirin reduced mortality in LASV infected NHPs [20••]. Ribavirin treatment in LF patients or in combination with convalescent plasma in NHPs has also been shown to reduce mortality rates [21••,22]. In accordance with these preliminary data, ribavirin has been recommended for 'off-label' use to treat LASV patients. These data are in contrast to more recent pre-clinical animal findings suggesting ribavirin may be of limited clinical use in an outbreak setting. An assessment of ribavirin's efficacy in the chimeric IFNAR$^{-/-}$ mouse model showed no improvement during LASV infection at a dose of 80 mg/kg/day and modest improvement at twice that dose [23]. A dose of 50 mg/kg/day in Hartley guinea pigs challenged with GPA-LASV was able to significantly delay death, but ultimately did not improve overall survival [14••]. These animals did not show any significant difference in temperature or weight change during the course of ribavirin treatment and began to show signs of disease following cessation of ribavirin therapy suggesting that continuation may improve survival. Treatment studies with ribavirin in NHPs have been limited since the 1980's when it was shown that rhesus macaques treated with ribavirin at the onset of LASV infection or five days post infection (dpi) could be fully protected although animals treated 5 dpi had more severe disease [20••]. Cynomolgus macaques were also protected against lethal LASV challenge if ribavirin administration was given early in infection, either at 0 or 4 dpi [22]. The utility of ribavirin in this context is difficult to determine given the long incubation period of LF and the determination of initial exposure. Despite its frequent use as a prophylactic measure against LASV, there remain limited animal and human data on the efficacy of ribavirin for protection against LF. Studies that have shown efficacy have used intravenous, intramuscular, or subcutaneous routes of ribavirin treatment as opposed to oral administration that is typically seen in post-exposure prophylaxis [24]. Questions remain regarding the pharmacological activity of ribavirin in this context. In addition, smaller scale studies in humans have also been done, but these are often uncontrolled, retrospective case studies with small sample sizes and unreliable adherence [25]. Overall, while there is evidence that ribavirin may have some efficacy against LASV, several pre-clinical issues remain to be resolved.

Favipiravir

The broad-spectrum antiviral compound, Favipiravir (T-705; 6-fluoro-3-hydroxy-2-pyrazinecarboxamide) is an RNA polymerase inhibitor, originally developed as an anti-influenza drug in Japan. It has recently undergone phase-3 clinical trials for treatment of Influenza [26]. Favipiravir has significant potential inhibitory action against RNA viruses including LASV. High doses of Favipiravir (300 mg/kg/day) given intraperitoneally (IP) starting 4 dpi can prevent lethality in the chimeric mouse model of LASV infection [23]. A lower dose of 150 mg/kg/day given in combination with ribavirin can also significantly improve survival [23]. In Guinea pigs, subcutaneous treatment with Favipiravir at either 150 or 300 mg/kg/day starting 2 dpi significantly increased survival rates compared to ribavirin treatment and reduced the viral burden in tissues [14••]. Further investigation showed that treatment with Favipiravir could be delayed to 7 DPI with 100% survival and 9 DPI with 83% survival [14••]. Additionally, an intravenous loading dose of 300 mg/kg given 4 dpi followed by daily subcutaneous injections were able to protect Cynomolgus macaques from lethal LASV infection. However, reducing the daily treatment to 150 mg/kg/day through three separate doses of 50 mg/kg did not improve disease outcome [27•]. Recently Favipiravir has also been administered in combination with Ribavirin to patients with LF, but it is difficult to determine its efficacy without controlled trials [25]. Given the drug's safety and approval for use in Japan, its use for treatment of LASV, either alone or in

Table 2

Antiviral therapeutics for the treatment of LASV infection

Treatment	Model	Challenge strain	Dose and route	Treatment dose (days)	Survival	Ref.
Ribavirin	Chimeric IFNAR$^{-/-}$	Ba366	10^3 FFU i.p.	80 mg/kg/day (4-death) i.p.	0%	[23]
				160 mg/kg/day (4–11) i.p.	20%	
	Hartley GP	GPA-Josiah	10^5 $TCID_{50}$ i.p.	50 mg/kg/day (2-death) s.c.	0%	[14••]
	Strain 13 guinea pig	Josiah	10^3 PFU s.c.	25 mg/kg/day (0–14) i.p.	0%	[20••]
	Rhesus macaque	Josiah	$10^{6.1}$ PFU s.c.	50 mg/kg loading, 10 mg/kg/day (0–18) s.c.	100%	[20••]
				50 mg/kg loading, 10 mg/kg/day (5–18) s.c.	100%	
				75 mg/kg loading, 30 mg/kg/day (0–18) i.m.	100%	
				75 mg/kg loading, 30 mg/kg/day (4–18) i.m.	100%	
	Cynomolgus macaque	Josiah	$10^{6.1}$ PFU s.c.	75 mg/kg loading, 30 mg/kg/day (7–18) i.m.	50%	[22]
				150 mg/kg loading, 60 mg/kg/day (7–18) i.m.	25%	
				225 mg/kg loading, 90 mg/kg/day (7–18) i.m.	0%	
Favipiravir	Chimeric IFNAR$^{-/-}$	Ba366	10^3 FFU i.p.	75 mg/kg/day (4-death) oral	0%	[23]
				150 mg/kg/day (4-death) oral	0%	
				300 mg/kg/day (4–11) oral	100%	
	Hartley GP	GPA-Josiah	10^5 $TCID_{50}$ i.p.	150 mg/kg/day (2–15) s.c.	83%	[14••]
				300 mg/kg/day (2–15) s.c.	100%	
				300 mg/kg/day (5–18) s.c.	100%	
				300 mg/kg/day (7–20) s.c.	100%	
				300 mg/kg/day (9–22) s.c.	83%	
	Cynomolgus macaque	Josiah	10^4 $TCID_{50}$ i.m.	300 mg/kg i.v. loading, 300 mg/kg/day s.c. (4–17)	100%	[27•]
				300 mg/kg i.v. loading, 150 mg/kg/day s.c. (4–17)	0%	
ST-193	Strain 13 guinea pig	Josiah	10^3 PFU s.c.	25 mg/kg/day (0–14) i.p.	62.50%	[29]
				80 mg/kg/day (0–14) i.p.	62.50%	
Immune plasma	Strain 13 guinea pig	Josiah, Z-132	3400 PFU s.c.	Guinea pig plasma		[34]
			4000 PFU s.c.	Rhesus plasma	0–100%	
				Human plasma		
	Cynomolgus macaque	Josiah	$10^{6.1}$ PFU s.c.	1 mL/kg (0, 3, 6)	87.50%	[22]
				1 mL/kg (4, 7, 10)	66.60%	
				1 mL/kg (7, 10, 13)	16.70%	
Monoclonal antibodies	Hartley guinea pig	Josiah	10^3 PFU i.p.	30 mg/kg/day (0, 3, 6) i.p.	0–100%	[37••]
				Single MAb 15 mg/kg (0, 4, 8) i.v.	100%	
				Single MAb 15 mg/kg (0, 5) i.v.	75%	
	Cynomolgus macaque	Josiah	3500 PFU i.m.	Mab cocktail 15 mg/kg (0, 4, 8)	100%	[38••]
				Mab cocktail 15 mg/kg (3, 6, 9)	100%	
				Mab cocktail 15 mg/kg (6, 9, 12)	100%	
				Mab cocktail 15 mg/kg (8, 11, 14)	100%	

combination with other antivirals or specific therapeutics should be examined further. Studies determining its efficacy against LASV and other haemorrhagic fevers are warranted with the recommendation of human trials by the US National Institute of Allergy and Infectious Disease.

Other antiviral drugs

ST-193 is a benzimidazole derivative that was identified through screening of drugs that could inhibit Arenavirus entry [28]. In a guinea pig model of LF it has been shown to be superior to ribavirin treatment when administered i.p; however, treatment began on the same day as LASV infection [29]. Its efficacy when given later in infection has yet to be described and is critical to further assess its candidacy as an effective therapeutic. A number of potential therapeutic options against LASV and other haemorrhagic fever viruses have been described and tested *in vitro* including LHF-535, an analog of ST-193 as well other candidates such as small interfering RNA and small molecules that inhibit viral fusion, entry, or replication [30–32]. Arbidol is a small molecule that is approved for treatment of Influenza in China and Russia and has shown *in vitro* efficacy against LASV [32]. Screens of FDA-approved drugs have identified other candidates that can prevent or reduce LASV infectivity *in vitro*;

however, most of these candidates need further development and testing *in vivo*.

Immunotherapy for LASV infection

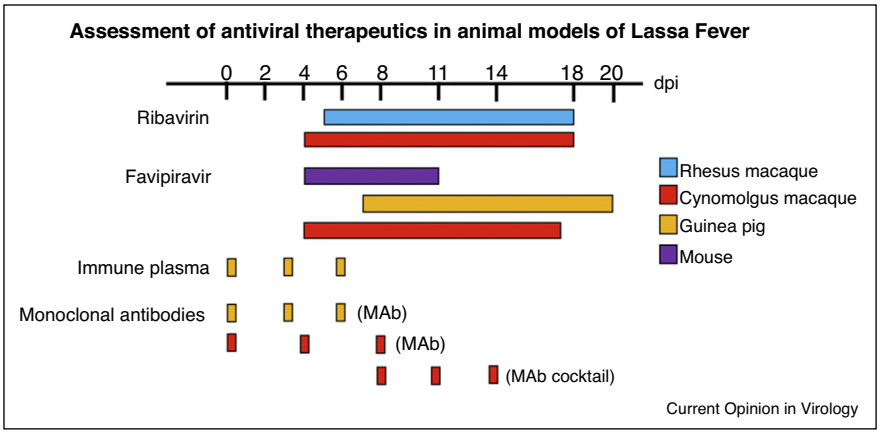
The use of immunoglobulin therapies in treating viral haemorrhagic fevers has taken off since the successful treatment of Ebola virus with ZMAPP [33]. While the role of humoral immunity in protection against LASV has been debated, there is some evidence that treatment with convalescent plasma or with monoclonal antibodies can prevent LF. Convalescent plasma has been used in animal models to treat LF and also been given to LF patients for decades. In an early study, guinea pigs treated with immune plasma obtained from convalescent guinea pigs, NHPs, or humans could fully protect against LF provided the amount of plasma and the neutralizing titers of the plasma were sufficient [34]. Successful treatment of LF in NHPs has also been achieved provided that immune serum is administered no later than 4 dpi [22]. It appears that the critical factor for treatment of LF with convalescent plasma is its neutralization capacity, as studies have shown that a $\log_{10}$ neutralization index of greater than two can be protective in guinea pigs and NHPs [22,35]. In the 1980's, the use of plasma for treatment of LF showed mixed results. In one study, despite low numbers and a low fatality rate in the control group, treatment before the tenth day of illness resulted in only a single death, while treatment after the tenth day saw 8/11 probable and possible LF cases result in death [36]. In another, plasma therapy was compared against treatment with ribavirin and in all cases treatment with plasma alone did not significantly improve survival [21••].

Treatment of LF with monoclonal antibodies has recently become an area of interest. A panel of human monoclonal antibodies screened for their ability to bind and neutralize LASV were tested in the guinea pig model of LF [37••]. Five antibodies were able to provide complete protection when administered on the date of challenge, while several others were able to significantly improve survival compared with control antibodies [37••]. Treatment of Cynomolgus macaques with single monoclonal antibodies or a cocktail of monoclonal antibodies was shown to provide complete protection against LF when administered on 0, 3, 6, and even 8 dpi; at a time when animals were viremic [38••]. This is similar to what is seen in NHP models of Ebola, where the ZMapp cocktail administered in the late stages disease is fully protective [39]. A limitation of this treatment option might be the requirement of antibodies of sufficiently high neutralizing titers; however, recent advances in molecular techniques for identifying and purifying monoclonal antibodies should ease this burden. This provides strong evidence for the utility of monoclonal antibody treatment several days after the onset of LF.

Conclusions and future considerations

Because of the significant burden of LF in West Africa, the development of effective therapeutic countermeasures will be critical for reducing the impact of the disease going forward. With no therapeutics or drugs approved for use in LF patients, there is an urgent need for testing and development of new and/or previously used drug options. Pre-clinical testing of anti-viral drugs and specific therapeutics requires reliable animal models to assess efficacy. Here we have outlined the animal models that have been developed and used for pathogenesis and therapeutic studies. While several different therapeutic options have been tested in pre-clinical studies as well as retrospective trials, much remains unknown regarding the correct approach for the treatment of LF (Figure 1). Ribavirin has been historically used, but its efficacy has

Figure 1



Assessment of antiviral therapeutics in animal models of Lassa fever.
Each of the antiviral therapeutics listed has shown 100% protective efficacy in mice, guinea pigs, or NHPs when treatment was administered at the day listed relative to infection. Extended bars indicate daily treatments (Ribavirin, Favipiravir) whereas short bars indicated single doses on the days indicated (Immune plasma, Monoclonal antibodies).

been called into question, and other options such as Favipiravir and monoclonal antibodies, have been shown to provide superior protection in pre-clinical models. Continued development of broad-spectrum antiviral drugs and immunotherapies is likely the best way to treat LF moving forward. The models and treatment options listed here will provide a strong framework from which to start the process of developing more efficacious therapeutic candidates for the treatment of LF.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- •• of outstanding interest

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